Bilirubin metabolism

An understanding of the mechanisms involved in the production of jaundice requires a knowledge of bilirubin metabolism. As advances have been made in this field it has become necessary to re-examine and modify some of the earlier concepts of jaundice. In recent years the most fruitful research has been that directed towards the elucidation of the origin and formation of bilirubin, and also investigations into the mechanisms of uptake, conjugation, and secretion of bilirubin by the liver cell; particular attention has been paid to the way in which these processes can be altered by drugs.

Bilirubin is primarily a breakdown product of haemoglobin, haem, from senescent red blood cells. The sequence of events in this process has been a controversial matter and has been discussed in detail by With.¹ The recent studies by Tenhunen *et al* (1968),² in which they demonstrated the enzymatic conversion of haem to bilirubin by haem oxygenases present in liver and spleen microsomes, can be expected to shed further light on this problem. Studies *in vivo* have suggested that haematin,³ protoporphyrin,⁴ and biliverdin⁵ may be intermediate metabolites. Normally 200 to 300 mg of bilirubin is produced each day and the human body is able to excrete this amount efficiently. Greater loads than this can be handled without clinical jaundice becoming apparent, and in some haemolytic states as much as 2,000 to 3,000 mg may be excreted each day with only a moderate elevation of the serum unconjugated bilirubin concentration, provided that liver function is normal.

Following the demonstration by London *et al* (1950)⁶ and Gray *et al* (1950)⁷ that some of the bilirubin produced each day (10%-20% in the normal subject) was derived from sources other than the haemoglobin-haem of senescent red cells, much attention has been given to this so-called 'early labelled bilirubin', and the possibility that an increase in its production might be a cause of jaundice has been explored (see review by Robinson, 1968⁸). Increased production of early labelled bilirubin has been documented in a wide range of disorders many, but not all, of which are characterized by some abnormality of erythropoietic function. A rational basis for these findings was provided by the studies of Israels and his colleagues,^{9,10} who found that early labelled bilirubin had two major components, one of which was related to erythropoiesis while the other was independent of erythropoiesis. There is now further information to suggest that each component comprises two or more subcomponents.¹¹

The non-erythropoietic component appears to be synthesized mainly in the liver, and, according to Levitt *et al* (1968),¹¹ is derived from 'free' haem or its precursors and also from the rapid turnover of several haem

proteins, including some of the microsomal cytochromes associated with drug metabolism. This latter component has been shown to be increased by phenobarbital administration in animals,¹² but the findings in one patient with congenital non-haemolytic hyperbilirubinaemia were equivocal.¹³ Increased formation of non-erythropoietic early labelled bilirubin has been reported in pernicious anaemia,¹⁰ in congenital erythropoietic porphyria,¹⁴ and in the Crigler-Najjar syndrome.¹³

The erythropoietic component is probably formed in the bone marrow, although its exact source is not currently known. It may arise from reticulocytes and normoblasts that fail to reach maturation (or haemolyse), inclusion bodies (especially in thalassaemia), and non-haemoglobin sources of haem or its precursor pyrroles.⁸ It is greatly increased by hyperplastic reactions of the bone marrow and may contribute to the hyperbilirubinaemia of many haemolytic disorders. Robinson *et al*¹⁵ have shown that the jaundice in a young girl with thalassaemia minor could be entirely accounted for by the formation of early labelled bilirubin.

The process whereby bilirubin is transferred from the plasma into the liver cell is still poorly understood. It appears to be extremely rapid and involves first the detachment of the pigment from albumin at the cell surface and then, according to Levi and his associates,¹⁶ acceptance by two specific binding proteins (Y and Z) in the cytoplasm of the cell. Such drugs as bunamiodyl¹⁷ and male fern oil (flavaspidic acid),¹⁸ which inhibit the hepatic uptake of bilirubin, probably act by competing with bilirubin for these binding sites. Bromsulphthalein is also bound by the Y and Z fractions, and, since its rate of removal from the plasma is not impaired in Gilbert's syndrome, it is unlikely that a deficiency of these proteins will prove to be responsible for the limited hepatic uptake of bilirubin in this condition.¹⁹

Although small amounts of bilirubin sulphate²⁰ have been identified in human bile there is little doubt that more than 90% of the bilirubin present in bile is in the form of its glucuronide derivatives. Glucuronide conjugation takes place in the endoplasmic reticulum of the liver cell with uridine diphosphoglucuronic acid as the glucuronyl donor and bilirubin UDP-glucuronyl transferase acting as the enzyme for the reaction. A deficiency of this enzyme has been convincingly demonstrated in the jaundice of prematurity²¹ and in the Crigler-Najjar syndrome²² and results in a severe unconjugated hyperbilirubinaemia. In the latter condition isotopic studies by Schmid and Hammaker²³ have shown that a steady serum bilirubin concentration is achieved by catabolism of the pigment and possibly excretion of bilirubin across the intestinal wall. Evidence regarding the role of the enzyme in a variety of conditions characterized by small or moderate increases in the serum unconjugated bilirubin has been conflicting, probably because of lack of sufficiently sensitive assay techniques. Recent studies, in which a new procedure for assaying bilirubin UDP-glucuronyl transferase activity in

specimens of liver obtained by needle biopsy was used, showed that enzyme activity was reduced in patients with Gilbert's syndrome and Wilson's disease, but not in other types of liver disease.²⁴ These results suggested that the impaired hepatic uptake of bilirubin in Gilbert's syndrome¹⁹ could be partially explained by the enzyme deficiency, since conjugation appears to be one of the limiting factors in this process. Interference with conjugation has been postulated as the cause of the unconjugated hyperbilirubinaemia which may follow the administration of some drugs, *eg*, novobiocin,²⁵ especially in the newborn.

The glucuronide conjugating capacity of the liver can be enhanced by the administration of drugs such as phenobarbital, which cause induction of microsomal enzymes.²⁶ Such treatment has resulted in reduction in the jaundice of patients with severe idiopathic unconjugated hyperbilirubinaemia,^{27,28} and probably represents the first therapeutic application of enzyme induction. Arias and his associates²⁹ have found that in the more severely affected patients with the Crigler-Najjar syndrome, whose bile is essentially colourless and who presumably have an absolute deficiency of the enzyme, no beneficial effect is obtained with phenobarbital. There is evidence that factors other than increased conjugation of bilirubin may be involved in this response,^{28,30} which might explain the improvement observed in some patients with biliary cirrhosis.³¹ Phenobarbital therapy does not, however, influence the degree of jaundice in patients with complete biliary obstruction.³² Whether its administration will prove of use in preventing severe jaundice in the newborn³³ remains to be established; in this situation the speed at which enzyme induction is achieved will be extremely important, so that it is possible that drugs other than phenobarbital may prove more beneficial.

The secretion of bilirubin into the bile requires the transfer of conjugated bilirubin across the membrane separating the liver cell from the lumen of the biliary canaliculus by an active transport mechanism, which is common for many organic anions, including bromsulphthalein, but not for conjugated bile acids.³⁴ Competition for this transport mechanism can be demonstrated by the administration of a variety of steroids, including the C-17 alkyl-substituted testosterone, and some of the progesterone and oestrogen components of the contraceptive pill.³⁵ In patients with the Dubin-Johnson or Rotor syndromes,³⁶ as well as in the Corriedale sheep,³⁴ the functioning of this transport system is impaired and a mild chronic conjugated hyperbilirubinaemia results without other features of cholestasis.

In the gut, bacterial conversion of bilirubin to urobilinogen compounds occurs.³⁷ Elegant isotopic studies by Lester and Schmid confirmed that there is an enterohepatic circulation for both bilirubin³⁸ and urobilinogen.³⁹ It is doubtful whether these processes are of physiological importance in the normal subject, although the detection of increased amounts of urobilinogen in the urine remains a useful diagnostic test in haemolytic disorders and liver disease.⁴⁰

In complete biliary obstruction the serum bilirubin concentration rises and then remains at a relatively constant level, largely as the result of renal excretion of the conjugated pigment.⁴¹ Recent studies have established that this process involves glomerular filtration of a small dialysable fraction of the plasma conjugated bilirubin rather than tubular secretion^{42,43} or non-ionic diffusion.⁴⁴

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REFERENCES

- ¹With, T. K. (1968). *Bile Pigments: Chemical, Biological and Clinical Aspects*. Chapter 11. Academic Press, New York.
- ²Tenhunen, R., Marver, H. S., and Schmid, R. (1968). The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. *Proc. nat. Acad. Sci. (Wash.)*, **61**, 748-755.
- ³Snyder, A. L., Schmid, R. (1965). The conversion of hematin to bile pigment in the rat. *J. Lab. clin. Med.*, **65**, 817-824.
- ⁴Ibrahim, G. W., Schwartz, S., and Watson, C. J. (1966). The conversion of protoporphyrin C¹⁴ to heme compounds and bilirubin in dogs. *Metabolism*, **15**, 1120-1128.
- ⁵Goldstein, G. W., and Lester, R. (1964). Reduction of biliverdin-C¹⁴ to bilirubin C¹⁴ *in vivo*. *Proc. Soc. exp. Biol. (N.Y.)*, **117**, 681-683.
- ⁶London, I. M., West, R., Shemin, D., and Rittenberg, D. (1950). On the origin of bile pigment in normal man. *J. biol. Chem.*, **184**, 351-358.
- ⁷Gray, C. H., Neuberger, A., and Sneath, P. H. A. (1950). Studies in congenital porphyria. II. Incorporation of ¹⁴N in the stercobilin in the normal and in the porphyric. *Biochem. J.*, **47**, 87-92.
- ⁸Robinson, S. H. (1968). The origins of bilirubin. *New Engl. J. Med.*, **279**, 143-149.
- ⁹Israels, L. G., Yamamoto, T., Skanderbeg, J., and Zipursky, A. (1963). Shunt bilirubin; evidence for two components. *Science*, **139**, 1054-1055.
- ¹⁰Yamamoto, T., Skanderbeg, J., Zipursky, A., and Israels, L. G. (1965). The early-appearing bilirubin evidence for two components. *J. clin. Invest.*, **44**, 31-41.
- ¹¹Levitt, M., Schachter, B. A., Zipursky, A., and Israels, L. G. (1968). The nonerythropoietic component of early bilirubin. *J. clin. Invest.*, **47**, 1281-1294.
- ¹²Schmid, R., Marver, H. S., and Hammaker, L. (1966). Enhanced formation of rapidly labeled bilirubin by phenobarbital; hepatic microsomal cytochromes as a possible source. *Biochem. biophys. Res. Commun.*, **24**, 319-328.
- ¹³Robinson, S. H., Lester, R., Crigler, J. F. Jr, and Tsong, M. (1967). Early labeled peak of bile-pigment in man. *New Engl. J. Med.*, **277**, 1323-1329.
- ¹⁴Gray, C. H., Kulczycka, A., Nicholson, D. C., Magnus, I. A., and Rimington, C. (1964). Isotope studies on a case of erythropoietic protoporphyria. *Clin. Sci.*, **26**, 7-15.
- ¹⁵Robinson, S., Vanier, T., Desforges, J. F., and Schmid, R. (1962). Jaundice in thalassaemia minor; a consequence of 'ineffective erythropoiesis'. *New Engl. J. Med.*, **267**, 523-529.
- ¹⁶Levi, A. J., Gatmaitan, Z., and Arias, I. M. (1968). Two cytoplasmic proteins from rat liver and their role in hepatic uptake of sulfobromophthalein (BSP) and bilirubin. *J. clin. Invest.*, **47**, 61a.
- ¹⁷Berthelot, P., and Billing, B. H. (1966). Effect of bumamiodyl on hepatic uptake of sulfobromophthalein in the rat. *Amer. J. Physiol.*, **211**, 395-399.
- ¹⁸Hammaker, L., and Schmid, R. (1967). Interference with bile pigment uptake in the liver by flavaspidic acid. *Gastroenterology*, **53**, 31-37.
- ¹⁹Billing, B. H., Williams, R., and Richards, T. G. (1964). Defects in hepatic transport of bilirubin in congenital hyperbilirubinaemia; an analysis of plasma bilirubin disappearance curves. *Clin. Sci.*, **27**, 245-257.
- ²⁰Noir, B. A., Groszman, R. J., and de Walz, A. T. (1966). Studies on bilirubin sulphate. *Biochim. biophys. Acta (Amst.)*, **117**, 297-304.
- ²¹Lathe, G. H., and Walker, M. (1958). The synthesis of bilirubin glucuronide in animal and human liver. *Biochem. J.*, **70**, 705-712.
- ²²Szabó, L., Kovács, Z., and Ébrey, P. B. (1962). Crigler-Najjar's syndrome. *Acta paediat. Acad. Sci. hung.*, **3**, 49-70.
- ²³Schmid, R., and Hammaker, L. (1963). Metabolism and disposition of C¹⁴-bilirubin in congenital non-hemolytic jaundice. *J. clin. Invest.*, **42**, 1720-1734.
- ²⁴Black, M., and Billing, B. H. (1969). Hepatic bilirubin UDP-glucuronyl transferase activity in liver disease and Gilbert's syndrome (in preparation).
- ²⁵Hargreaves, T., and Holton, J. B. (1962). Jaundice of the newborn due to novobiocin. *Lancet*, **1**, 839.
- ²⁶Catz, C., and Yaffe, S. J. (1968). Barbiturate enhancement of bilirubin conjugation and excretion in young and adult animals. *Pediatr. Res.*, **2**, 361-370.
- ²⁷Yaffe, S. J., Levy, G., Matsuzawa, T., and Baliah, T. (1966). Enhancement of glucuronide-conjugating capacity in a hyperbilirubinemic infant due to apparent enzyme induction by phenobarbital. *New Engl. J. Med.*, **275**, 1461-1466.
- ²⁸Whelton, M. J., Krustev, L. P., and Billing, B. H. (1968). Reduction in serum bilirubin by phenobarbital in adult unconjugated hyperbilirubinaemia. Is enzyme induction responsible? *Amer. J. Med.*, **45**, 160-164.
- ²⁹Arias, I. M., Gartner, L. M., Cohen, M., Ben-Ezzer, J., and Levi, A. J. (1968). Chronic nonhaemolytic unconjugated hyperbilirubinaemia with glucuronyl transferase deficiency; evidence for genetic heterogeneity. *Trans. Ass. Amer. Phycns.*, (in press).
- ³⁰Crigler, J. F., and Gold, N. I. (1966). Sodium phenobarbital-induced decrease in serum in an infant with congenital nonhemolytic jaundice and kernicterus. *J. clin. Invest.*, **45**, 998-999.
- ³¹Thompson, R. P. H., and Williams, R. (1967). Treatment of chronic intrahepatic cholestasis with phenobarbitone. *Lancet*, **2**, 646-648.

- ³²Armas-Merino, R., and Whelton, M. (unpublished observations).
- ³³Maurer, H. M., Wolff, J. A., Ginster, M., Poppess, P. J., Pantuck, E., Kuntzman, R., and Conney, A. H. (1968). Reduction in concentration of total serum bilirubin in offspring of women treated with phenobarbitone during pregnancy. *Lancet*, **11**, 122-124.
- ³⁴Cornelius, C. E., Arias, I. M., and Osburn, B. I. (1965). Hepatic pigmentation with photosensitivity. A syndrome in Corriedale sheep resembling Dubin-Johnson syndrome in man. *J. Amer. vet. Med. Ass.*, **146**, 709-713.
- ³⁵Mulligan, R., and Sherlock, S. (1968). The effects of oral contraceptives on hepatic function. *Clin. Trials J.*, **5**, suppl. 155-164.
- ³⁶Gartner, L. M., and Arias, I. M. (1968). Pharmacologic and genetic determinants of disordered bilirubin transport and metabolism in the liver. *Ann. N. Y. Acad. Sci.* **151**, 833-841.
- ³⁷Watson, C. J. (1963). Recent studies of the urobilin problem. *J. clin. Path.*, **16**, 1-11.
- ³⁸Lester, R., and Schmid, R. (1963). Intestinal absorption of bile pigments. II. Bilirubin absorption in man. *New Engl. J. Med.*, **269**, 178-182.
- ³⁹——, Schumer, W., and Schmid, R. (1965). Intestinal absorption of bile pigments IV. Urobilinogen absorption in man. *Ibid.*, **272**, 939-943.
- ⁴⁰Bernstein, R. B., Troxler, R. F., and Lester, R. (1968). The effect of hepatobiliary disease on urobilinogen excretion. *Gastroenterology*, **54**, 150.
- ⁴¹Wollaeger, E. E., and Gross, J. B. (1966). Complete obstruction of the extrahepatic biliary tract due to carcinoma as determined by the fecal urobilinogen test. *Medicine (Baltimore)*, **45**, 529-536.
- ⁴²Fulop, M., Sandson, J., and Brazeau, P. (1965). Dialysability, protein binding, and renal excretion of plasma conjugated bilirubin. *J. clin. Invest.*, **44**, 666-680.
- ⁴³Ali, M. A. M., and Billing, B. H. (1963). Renal excretion of bilirubin by the rat. *Amer. J. Physiol.*, **214**, 1340-1345.
- ⁴⁴——, ———. (1966). Effect of acid-base changes on renal clearance of bile pigments. *Clin. Sci.*, **30**, 543-552.